Programmed Cell Death (apoptosis)

Stereotypic death process includes:
- membrane blebbing
- nuclear fragmentation
- chromatin condensation and DNA fragmentation
- loss of mitochondrial integrity and release of cytochrome c

Natural part of development
eg., removal of webbing between digits

Cancer involves mutations that block apoptosis (p53)

First genes discovered in nematodes
Self-fertile hermaphrodites; rapid life cycle
Early Embryogenesis: making founder cells
Six Founder Cells
(5 Somatic, 1 Germline)
C. elegans reproduces sexually

Hermaphrodite

self-fertile: germline makes ~300 sperm then oocytes

Male

cross-fertile: germline makes sperm only
C. elegans development

John Sulston’s drawings of nuclear positions (6 May 1980)
An invariant cell lineage

959 somatic cells (adult hermaphrodite)

(John Sulston, Bob Horvitz, Judith Kimble 1977-1983)
Caenorhabditis elegans Cell Lineage

Embryonic

Larval

The MS Lineage:

pharyngeal cell paapaaa

cell death paapp

8-cell stage

MS
Programmed Cell Death in C. elegans:

Embryogenesis produces a hatched larva with
--558 living cells
--131 cells eliminated by programmed cell death
(Shortly after their births).

Additional cell deaths occur during larval development.

Most cell deaths are in neuronal lineages.

Cell death (apoptosis) conserved in most animals.
Cancer connection.
**ced mutants:**
programmed cell death-defective

Ed Hedgecock: unbiased Nomarski screen for mutants with defects in cellular anatomy (F2 screens)

Identified two mutants, *ced-1* and *ced-2*, with persistent cell death corpses.
ced mutant microscopy

ced-1(-/-) and ced-2(-/-):

corpses accumulate
Cell death corpse accumulation: an entry point into genetic studies of programmed cell death, but not what you want to study.

What genes are REQUIRED for programmed cell death???
Mutants lacking programmed cell death (not cleaning up the mess).

Take advantage of ced-1/2 mutant phenotype: easy to see that programmed cell death is occurring.

Screen for mutants in which no corpses are visible (in a ced-1/2 mutant background)
**ced3 mutants fail to accumulate corpses**

*Horvitz lab rides again.*

Ellis et al, Cell **44**, 817-829 (1986)

**ced-1(-/-)**

**ced-1(-/-); ced-3(-/-)**

Figure 1. Absence of Cell Deaths in ced-3 Animals
Screens for mutants identify 2 genes

Screen: 4000 F1 broods examined
2 alleles of ced-3 identified
1 allele of ced-4 identified in different screen (Egl sup)

Not an easy screen, but succeeded in identifying two genes required for ALL cell deaths.

First time such genes found in any organism.

Figure 2. A Partial Genetic Map of Linkage Groups III and IV
Quantitation of cell deaths in *ced-3* and *-4* mutants

Table 1. Elimination of Cell Death by *ced-3* and *ced-4* Mutations

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Average Number of Deaths Observed</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Embryonic Deaths</td>
<td>Postembryonic Deaths</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Head of L1</td>
<td>Ventral Cord*</td>
<td>Postdeirid†</td>
<td>Q‡</td>
<td></td>
</tr>
<tr>
<td><em>ced-1</em></td>
<td>28.0</td>
<td>8.7</td>
<td>0.93</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( n = 23 )</td>
<td>( n = 28 )</td>
<td>( n = 29 )</td>
<td>( n = 28 )</td>
<td></td>
</tr>
<tr>
<td><em>ced-1; ced-3 (n717)</em></td>
<td>0.3</td>
<td>0.04</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( n = 21 )</td>
<td>( n = 50 )</td>
<td>( n = 15 )</td>
<td>( n = 24 )</td>
<td></td>
</tr>
<tr>
<td><em>ced-1; ced-3 (n718)</em></td>
<td>0.5</td>
<td>0.03</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( n = 26 )</td>
<td>( n = 35 )</td>
<td>( n = 24 )</td>
<td>( n = 21 )</td>
<td></td>
</tr>
<tr>
<td><em>ced-1; ced-3 (n1040)</em></td>
<td>7.0$</td>
<td>0</td>
<td>0.05</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( n = 21 )</td>
<td>( n = 23 )</td>
<td>( n = 20 )</td>
<td>( n = 17 )</td>
<td></td>
</tr>
<tr>
<td><em>ced-1; ced-3 (n1129)</em></td>
<td>3.0</td>
<td>N.D.</td>
<td>0.13</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( n = 22 )</td>
<td></td>
<td>( n = 30 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ced-1; ced-4 (n1162)</em></td>
<td>0.6</td>
<td>0.04</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( n = 23 )</td>
<td>( n = 27 )</td>
<td>( n = 21 )</td>
<td>( n = 21 )</td>
<td></td>
</tr>
</tbody>
</table>
*ced-3* (and *-4*) are required for pcd in all lineages.
Model for *ced* gene order of action

- ** initiation of death
- ** dying cell
- ** ced-4
- ** ced-3

- ** engulfment
- ** pycnotic nucleus of dead cell
- ** ced-2
- ** ced-1

- ** engulfing cell
- ** nuc-1

- ** DNA degradation
Screened 40,000 F2s for extra pharyngeal cells
(at locations of I2 and NSN corpses)

-- Identified multiple recessive alleles of \textit{ces-2},
required only in pharynx for cell deaths.

-- One dominant allele of a gene called \textit{ced-9}, prevents ALL.
*ced-9 (n1950) is a dominant gain-of-function allele*
**ced-9(gof)** prevents pcd of HSN neurons also

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HSNs missing (%)</th>
<th>No of sides</th>
<th>Egg-laying defective (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (N2)</td>
<td>1</td>
<td>250</td>
<td>0.4</td>
<td>704</td>
</tr>
<tr>
<td>egl-1</td>
<td>99</td>
<td>200</td>
<td>99</td>
<td>447</td>
</tr>
<tr>
<td>ced-3; egl-1</td>
<td>0</td>
<td>160</td>
<td>0.2</td>
<td>599</td>
</tr>
<tr>
<td>ced-4; egl-1</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>417</td>
</tr>
<tr>
<td>ced-9(n1950); egl-1</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>417</td>
</tr>
</tbody>
</table>
Isolation of *ced-9* loss-of-function alleles

The process involves the use of EMS (mutagenesis) to create a population of mutants. Then, a cross is made between *egl-1(sd)* males and *ced-9(n1950 dm)* females. The resulting progeny are analyzed for the presence of the desired alleles.

- **Common:**
  - *egl-1(sd)* males are able to lay eggs.
  - *ced-9(n1950 dm)* females cannot lay eggs.

- **Rare:**
  - Specific crosses may yield egg-laying defective phenotypes.

The diagram illustrates the genetic interactions and the expected phenotypes based on the genetic crosses.
**ced-9** lof alleles:

*n1950n2077* behaves like a null; *n1950n2161* is a weaker allele

<table>
<thead>
<tr>
<th>Table 2: Phenotypes of ced-9(0) mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td><strong>(a) Sterility and maternal-effect lethality</strong></td>
</tr>
<tr>
<td>Eggs laid per animal</td>
</tr>
<tr>
<td>Hatching (%)</td>
</tr>
<tr>
<td>L1 arrest (%)</td>
</tr>
<tr>
<td><strong>(b) Egg-laying defect</strong></td>
</tr>
<tr>
<td>Egg-laying defective (%)</td>
</tr>
<tr>
<td>HSNs missing (%)</td>
</tr>
<tr>
<td><strong>(c) Absence of rays in male tails</strong></td>
</tr>
<tr>
<td>Rays per side</td>
</tr>
<tr>
<td><strong>Numbers of eggs laid by first generation ced-9(0) homozygotes.</strong></td>
</tr>
</tbody>
</table>
*ced-9 (lof)* causes ectopic cell deaths
What is the order of action of the *ced* genes?

Loss of CED-9 leads to all cells undergoing programmed cell death (all cells are poised to die, but for CED-9 all would!)

CED-3/4 required for programmed cell deaths

Does CED-9 inhibit CED-3/4 function to prevent programmed cell death?
**ced-3 and -4 mutations suppress ced-9 (lof) mutations**

### TABLE 3  Mutations in ced-3 and ced-4 suppress the defects resulting from ced-9 (lof)

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Eggs laid per animal</th>
<th>Viable progeny</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ced-9(+)</td>
<td>207 ± 36</td>
<td>207 ± 33</td>
<td>14</td>
</tr>
<tr>
<td>ced-9(n1950 n2077)</td>
<td>1.6 ± 1.4</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>ced-4 ced-9(n1950 n2077)</td>
<td>200 ± 19</td>
<td>160 ± 20</td>
<td>12</td>
</tr>
<tr>
<td>ced-4</td>
<td>182 ± 17</td>
<td>148 ± 17</td>
<td>12</td>
</tr>
</tbody>
</table>
ced function pathway

CED-9 → CED-3 → CED-4 → cell death

ON: cell survival
OFF: cell death
Overexpression of Ced-3 and Ced-4 causes ectopic cell death

Enables another genetic test of \textit{ced-9} relationship to \textit{ced-3} and \textit{-4}

Also lets us test the relationship between \textit{ced-3} and \textit{ced-4}
Overexpression of Ced-3 and Ced-4 causes ectopic cell death.

Table 1. Overexpression of ced-3 or ced-4 can kill the ALM neurons

<table>
<thead>
<tr>
<th></th>
<th>Percent surviving ALMs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no. ALMS/no. sides scored)</td>
</tr>
<tr>
<td>wild-type</td>
<td>100 (31/31)</td>
</tr>
<tr>
<td>$P_{mec-7lacZ}$</td>
<td>100 (40/40)</td>
</tr>
<tr>
<td>$P_{mec-7ced-3A}$</td>
<td>20 (9/46)</td>
</tr>
<tr>
<td>$P_{mec-7ced-3B}$</td>
<td>42 (16/38)</td>
</tr>
<tr>
<td>$P_{mec-7ced-3C}$</td>
<td>100 (48/48)</td>
</tr>
<tr>
<td>$P_{mec-7ced-4A}$</td>
<td>10 (4/39)</td>
</tr>
<tr>
<td>$P_{mec-7ced-4B}$</td>
<td>87 (33/38)</td>
</tr>
<tr>
<td>$P_{mec-7ced-4C}$</td>
<td>98 (39/40)</td>
</tr>
<tr>
<td>$P_{mec-7ced-4D}$</td>
<td>98 (40/41)</td>
</tr>
</tbody>
</table>
Loss of ced-9 enhances ectopic cell death caused by overexpression of Ced-3 and Ced-4

Table 2. Effects of ced-9 on killing by ced-3 or ced-4 overexpression

<table>
<thead>
<tr>
<th></th>
<th>Percent surviving ALMs (no. ALMs/no. sides scored)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ced-9: ced-3</td>
</tr>
<tr>
<td>A. ALM killing by ced-3 overexpression is better in a ced-9(lf) background^a</td>
<td></td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-3A}$</td>
<td>0 [0/29]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-3B}$</td>
<td>0 [0/37]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-3C}$</td>
<td>21 [9/43]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-3/4A}$</td>
<td>43 [16/37]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-3/4B}$</td>
<td>67 [18/27]</td>
</tr>
<tr>
<td></td>
<td>ced-4: ced-9</td>
</tr>
<tr>
<td>B. ALM killing by ced-4 overexpression is better in a ced-9(lf) background^b</td>
<td></td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-4A}$</td>
<td>0 [0/30]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-4B}$</td>
<td>53 [18/34]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-4C}$</td>
<td>42 [15/36]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-4D}$</td>
<td>15 [4/27]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-3/4A}$</td>
<td>70 [35/50]</td>
</tr>
<tr>
<td>$P_{mec.\gamma ced-3/4B}$</td>
<td>74 [37/50]</td>
</tr>
</tbody>
</table>
Ced-3 is required for ectopic killing caused by overexpression of Ced-4, but not vice versa.
Questions Geneticists Ask

Does the (recessive) mutation confer a null phenotype?

Compare phenotype of a diploid homozygous for the mutation to a diploid heterozygous for the mutation and for a deficiency

\[
geneX^-/geneX^- \quad \text{geneX}^-/Df
\]
Questions Geneticists Ask

Does the (dominant) mutation represent a gain-of-function or an instance of haploinsufficiency?

Compare phenotype of a diploid heterozygous for the mutation to a diploid heterozygous for a deficiency of the region

wildtype/mutation     wildtype/Df
Questions Geneticists Ask

Do the identified genes function in a linear pathway?

Compare phenotypes of two double mutant strains. For each gene, one needs alleles with contrasting phenotypes. In the case of *ced* genes, alleles that confer no cell death (*ncd*) and alleles that confer ectopic cell death (*ecd*) are available.

$ced-3^{ncd} \quad ced-4^{ecd} \quad ced-3^{ecd} \quad ced-4^{ncd}$
**ced-9** \[\rightarrow\] **ced-4** \[\rightarrow\] **cell death**

**ced-9** \[\rightarrow\] **ON** \[\rightarrow\] **cell survival**

**ced-9** \[\rightarrow\] **OFF** \[\rightarrow\] **cell death**

**ced-9** \[\rightarrow\] **ced-4** \[\rightarrow\] **ced-3** \[\rightarrow\] **cell death**
One more player, Egl-1

Gain-of-function *egl-1* mutations cause HSNs to undergo programmed cell death. (The Horvitz lab used these *egl-1* mutations to isolate some *ced* mutants.)

Loss-of-function *egl-1* mutations, isolated exactly as *ced-9 (lof)* alleles were isolated, prevent programmed cell death.

Epistasis experiments place *egl-1* upstream of all *ced* gene functions
**egl-1** induced ectopic killing is suppressed by mutations that block pcd

<table>
<thead>
<tr>
<th>Transgene</th>
<th>% ALMs Surviving (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{mec-7}$ A</td>
<td>98</td>
</tr>
<tr>
<td>$P_{mec-7}$ B</td>
<td>100</td>
</tr>
<tr>
<td>$P_{mec-7}$ egl-1 A</td>
<td>8</td>
</tr>
<tr>
<td>$P_{mec-7}$ egl-1 B</td>
<td>9</td>
</tr>
<tr>
<td>$P_{mec-7}$ egl-1 C</td>
<td>10</td>
</tr>
<tr>
<td>$P_{mec-7}$ egl-1 C/+</td>
<td>50</td>
</tr>
<tr>
<td>$P_{mec-7}$ egl-1 C; ced-9 (gf)</td>
<td>98</td>
</tr>
<tr>
<td>$P_{mec-7}$ egl-1 C; ced-4 (lf)</td>
<td>97</td>
</tr>
<tr>
<td>$P_{mec-7}$ egl-1 C; ced-3 (lf)</td>
<td>98</td>
</tr>
</tbody>
</table>
Biochemistry and cell biology of Ced proteins

1. Egl-1 and Ced-9 interact (IP experiments)

Radioactive Egl-1 was incubated with GST-Ced-9 (lane 1) or GST (lane 5) and bound proteins separated by electrophoresis.

The BH3 domain of Egl-1 is required; compare lanes 1 and 2.

Figure 3. EGL-1 and CED-9 Interactions
Biochem and cell biology, continued

2. Bunches of IP experiments demonstrate interaction between Egl-1 and Ced-9, between Ced-9 and Ced-4, and between Ced-3 and Ced-4

3. Ced-9 is localized to the mitochondrial outer membrane and recruits Ced-4

4. Induction of programmed cell death induces Ced-4 translocation to the nuclear membrane but not in gof Ced-9 mutants
Figure 1 CED-9 and CED-4 are localized to mitochondria in WT embryos.

F Chen et al. Science 2000;287:1485-1489
Figure 2 CED-9 is required for the localization of CED-4 to mitochondria.

F Chen et al. Science 2000;287:1485-1489
Figure 4 Overexpression of EGL-1 induces CED-4 translocation from mitochondria to nuclear membranes inced-9(+) embryos but not inced-9(n1950) embryos.

Ced-4 in wt embryos overexpressing Egl-1

Ced-4 in ced-9(gof) embryos overexpressing Egl-1

F Chen et al. Science 2000;287:1485-1489

Published by AAAS
Current model

A

SPECIFICATION

ces-2 → ces-1 → egl-1 → ced-9 → ced-4 → ced-3 → Cell Death

EXECUTION

NSM Sister Cells

All Dying Cells

B

CED-4

BGL-1

CED-4

→ Cell Death
Figure 3. Biochemical model for the activation of programmed cell death. (A) In living cells, CED-4 is tethered to the surface of mitochondria through binding to CED-9. (B) In cells that are doomed to die, the death initiator EGL-1 binds to CED-9, causes a major CED-9 conformational change, and triggers the disassociation of CED-4 from CED-9. (C) Released CED-4 proteins translocate to perinuclear membranes and undergo oligomerization, which brings two CED-3 proenzymes to close proximity. (D) CED-3 proenzymes undergo autoproteolytic activation.
Cloning *ced* genes revealed similarities to mammalian proteins

Ced-3 is similar to mammalian interleukin converting enzyme, a cysteine protease

Ced-3 is therefore proposed to be a cysteine protease

In vitro substrates for Ced-3 include actin, tubulin, and proteins involved in ATP synthesis and in DNA synthesis

Ced-9 is a homolog of Bcl-2, a mammalian oncogene
Comparison of apoptotic pathways

Caenorhabditis elegans

Healthy  
- Ced-9  
- Ced-4  

Apoptotic  
- Ced-9  
- Egl-1  

Survival  
- Ced-3  
- Csp-3  

Apoptosis  
- Ced-3  

Drosophila

Healthy  
- Debcl  

Apoptotic  
- Debcl  
- RHG  
- Diap1  
- Diap1  

Survival  
- Dark  
- Diap1  
- Dronc  
- Dronc  

Apoptosis  
- Dark  
- Dronc  

Vertebrate

Healthy  
- Bax  
- Bak  
- Bcl-2  

Apoptotic  
- Apaf-1  
- XIAP  
- XIAP  

Survival  
- Apaf-1  
- XIAP  
- XIAP  

Apoptosis  
- Apaf-1  
- XIAP  
- XIAP  

Legend:
- Csp-6/endoG
- Omi/HtrA2/dOmi
- Smac/DIABLO
- Wah-1/AIF
- Cyt c
- Activated form

Annu. Rev. Genet. 43:95–118
Ced-9 is a functional homolog of mammalian bcl-2
Over-expression of **bcl-2** mimics over-expression of **ced-9**
Parallels of CED-9 in worms and Bcl-2 in humans.

Bcl-2 is a human oncogene with properties similar to CED-9

Over-expression of Bcl-2 prevents or delays cell death in B-cell and T-cell lineages.

Bcl-2 expressed at high levels in blood stem cell lineages; loss of expression correlates with appearance of cell death

In cancer, chromosome translocations activate Bcl-2 expression, preventing cell death in hematopoietic lineages. This results in a leukemia due to over-proliferation of some blood cell lineages. No LOF alleles.
Comparison of apoptotic pathways

Annu. Rev. Genet. 43:95–118
3 subfamilies of Bcl-like proteins

1. Anti-apoptotic proteins, BH1-4 – BCL-2, BCL-xL, MCL-1, A1, BCL-w

2. Pro-apoptotic proteins, BH1-3 – BAX, BAK, BOK

3. Pro-apoptotic proteins, BH3-only – BIK, BID, BIM, BAD, PUMA, NOXA, HRK etc
Another look at the subfamilies

**Anti-apoptotic BCL-2 proteins**
- **BH4**
- **BH3**
- **BH1**
- **BH2**
- **TM**
- **α1**
- **α2**
- **α3**
- **α4**
- **α5**
- **α6**
- **α7**
- **α8**
- **α9**

**Pro-apoptotic BCL-2 proteins**
- **BH3**
- **BH1**
- **BH2**
- **TM**
- **α1**
- **α2**
- **α3**
- **α4**
- **α5**
- **α6**
- **α7**
- **α8**
- **α9**

**‘Effectors’**
- **BAK, BAX, (BOK*)**

**‘BH3-only’**
- **BH3**
- **TM**
- **BID, BIM, BAD, BIK, BMF, bNIP3, HRK, Noxa, PUMA**
Mouse mutants defective for Bcl-2 family members have altered cell death phenotypes

<table>
<thead>
<tr>
<th>BCL-2 family member</th>
<th>Defects caused by its deletion*</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pro-survival family members</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCL-2</td>
<td>Abnormal death of renal epithelial progenitors, melanocyte progenitors and mature B and T lymphocytes. Causes fatal polycystic kidney disease (100% mortality by 6 weeks), premature greying and lymphopenia (but all of these effects can be rescued by concomitant loss of the BH3-only protein BIM).</td>
<td>130</td>
</tr>
<tr>
<td>BCL-XL</td>
<td>Abnormal death of fetal erythroid progenitors and neuronal cells. Causes death around embryonic day 14 (100% mortality).</td>
<td>129</td>
</tr>
<tr>
<td>BCL-W</td>
<td>Abnormal death of developing sperm cells. Causes male sterility.</td>
<td>132</td>
</tr>
<tr>
<td>A1A</td>
<td>Abnormally accelerated death of granulocytes and mast cells in culture.</td>
<td>133</td>
</tr>
<tr>
<td>MCL1</td>
<td>Failure in implantation. Conditional knockout causes premature death of immature and mature B and T lymphoid cells, as well as haemopoietic stem cells.</td>
<td>128</td>
</tr>
<tr>
<td><strong>Pro-apoptotic BAX/BAK family members</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAX</td>
<td>Mild lymphoid hyperplasia, male sterility due to spermatocyte differentiation defect.</td>
<td>135</td>
</tr>
<tr>
<td>BAK</td>
<td>No obvious defects detected so far.</td>
<td>136</td>
</tr>
<tr>
<td><strong>Pro-apoptotic BH3-only proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIM</td>
<td>Lymphoid and myeloid cell hyperplasia, fatal SLE-like autoimmune disease (on mixed genetic C57BL/6x129Sv background), many cell types are abnormally resistant to cytokine deprivation, deregulated calcium flux and the chemotherapeutic drug taxol; mild but significant resistance of many cell types to DNA damage and glucocorticoids.</td>
<td>143</td>
</tr>
<tr>
<td>BID</td>
<td>BID-deficient mice are resistant to fas-activation-induced hepatocyte killing and fatal hepatitis; however, some cell types (such as lymphoid cells) are normally sensitive to fas-induced apoptosis.</td>
<td>13, 14</td>
</tr>
<tr>
<td>PUMA</td>
<td>Many cell types are profoundly resistant to DNA damage; many are also resistant to cytokine deprivation, glucocorticoids and phorbol esters.</td>
<td>150, 151</td>
</tr>
<tr>
<td>BAD</td>
<td>Mild resistance of some cell types to deprivation of epidermal growth factor or insulin growth factor.</td>
<td>154</td>
</tr>
<tr>
<td>HRK</td>
<td>Abnormal, although relatively mild, resistance of certain neuronal populations to deprivation of nerve growth factor.</td>
<td>155, 156</td>
</tr>
<tr>
<td>BIK</td>
<td>No obvious defects detected so far.</td>
<td>158</td>
</tr>
<tr>
<td>NOXA</td>
<td>Relatively mild resistance of fibroblasts to γ-irradiation or etoposide, but profound resistance of these same cells and keratinocytes in the skin to ultraviolet irradiation.</td>
<td>150</td>
</tr>
</tbody>
</table>

*These are phenotypes found in mice. The roles of these proteins may differ in humans. BAD, BCL-2 antagonist of cell death; BAK, BCL-2 antagonist/killer-1; BAX, BCL-2-associated X protein; BCL-2, B-cell lymphoma-2; A1A, BCL-2-related protein A1A; BCL-W, BCL-2-like-2; BCL-XL, a BCL-2-like protein; BID, BH3-interacting domain death agonist; BIK, BCL-2-interacting killer; BIM, BCL-2-like-1; HRK, harakiri (also known as death protein-5); MCL1, myeloid cell leukaemia sequence-1; PUMA, BCL-2 binding component-3; SLE, systemic lupus erythematosus.
A model for mammalian cells
Same model, a little more detail
Protein localization in dividing cells

Some Bcl is localized to the mitochondrion

Bax is in the cytoplasm
Bax localization after induction of apoptosis
Figure 1 Resistance of Bax, Bak doubly deficient murine embryonic fibroblasts (MEFs) to tBID-induced apoptosis.

Quantitation of tBID induced apoptosis

GFP identifies infected cells (expressing tBID)

M C Wei et al. Science 2001;292:727-730
Figure 2 Function of BAX and BAK downstream of tBID and upstream of cytochrome c release.

Cytochrome c microinjection

M C Wei et al. Science 2001;292:727-730
Figure 4 Resistance of Bax, Bak doubly deficient MEFs to multiple intrinsic death signals.

M C Wei et al. Science 2001;292:727-730
BimS is a BH3-only protein

Bak and Bax are required for Bims-induced apoptosis
a Anti-apoptotic BCL-2 proteins

BH4 BH3 BH1 BH2 TM

BCL-2, BCL-W, BCL-XL, A1 and MCL-1

Pro-apoptotic BCL-2 proteins

Effectors

BH3 BH1 BH2 TM

BAK, BAX and BOK

BH3-only proteins

BH3

BID, BIM, BAD, BIK, BMF, BNIP3, HRK, NOXA and PUMA

b Indirect activator model

BH3-only protein

Anti-apoptotic BCL-2 protein

Active

BAX or BAK

Direct activator-derepressor model

Sensitizer

BH3-only protein

Anti-apoptotic BCL-2 protein

Active

BAX or BAK

Direct activator
Cytochrome c is required for oligomerization of Apaf and for activation of caspase.
CED-9 is BCL-2 (a negative regulator of Caspases)
Dominant mutation pays off (by way of lof).

CED-3 is a pro-caspase, while CED-4 is related to vertebrate Apaf-1 (caspase activator); both found to be required in all animal cells for apoptosis.

CED-9/BCL-2 are associated with mitochondrial membrane: how they are regulated and role of mitochondria remain subject of active research.

One goal: trigger cancer cells to all enter apoptosis.
Back to checkpoints

p53 is a target of checkpoint pathways and determines survival versus death

In death mode, p53 interacts with and causes oligomerization of Bak

This interaction causes release of cytochrome c from the mitochondrion

The model is that p53 and the Bcl-2 family member Mcl1 have opposing effects on the death effector, Bak